THE ROLE OF THE BLOOD IN THE TRANSPORTATION OF STRONTIUM^{90}–YTTRIUM^{90} IN TELEOST FISH [1, 2]

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As the result of global fallout and the introduction of radioactive wastes from nuclear reactor plants into the oceans, marine organisms are being subjected to an environment which is potentially hazardous to themselves and to other members of the ecosystems involved. During the last few years, a study has been made in this laboratory of various aspects of the metabolism of radiostrontium by marine fish. These fish may pick up strontium directly from sea water, by way of the skin, gills, or by swallowing the water (Boroughs, Townsley and Hiatt, 1956). They may also take up this element from their food. In any event, the transportation of strontium within the fish, including its excretion, depends upon its transportation by the blood, except for the strontium which is unabsorbed from the digestive tract.

It is the purpose of this paper to report on certain aspects of the transportation of strontium^{90}-yttrium^{90} in teleost blood.

Materials and Methods

The species used in this experiment was *Tilapia mossambica,* a teleost fish. Individuals weighed between 50 and 110 grams each. They were kept in tanks supplied with running sea water.

Two concentrations of Oak Ridge Sr^{90}-Y^{90} were prepared by dilution with saline solution approximately isotonic with *Tilapia* blood. Those fish which were to be bled a day or more after injection were given 100μc of Sr^{90}, while the fish killed at shorter time intervals were given only 10 μc. In both instances the dose injected was 0.2 ml.

The injections were made, and blood was withdrawn with the fishes' opercula in water. Separate fish were used for each time interval studied instead of using a single fish for repetitive bleedings. All the fish were handled as gently and uniformly as possible, and their eyes were covered with the hand. We believe this procedure results in a minimum of trauma.

The Sr^{90}-Y^{90} dose was injected directly into the ventricle of the heart. At predetermined time intervals of 5, 15, 30, and 45 minutes and 1, 4, and 8 days, as much as possible of each fish's blood was withdrawn through the kidney sinus. A red blood cell count was made each time a fish was injected and again when blood was removed.

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Immediately after removing the blood from the fish, triplicate 0.1-ml. samples were pipetted onto circles of one thickness of absorbent tissue on aluminum planchettes. Three-tenths-ml. aliquots of the remaining blood were centrifuged for 10 minutes at 2100 rpm in calibrated small bore hematocrit tubes in an International clinical centrifuge. The separated blood in one tube was used for measuring the radioactivity in the plasma and also that associated with the cells. From a second tube the plasma was removed without disturbing the packed cells. Five-hundredths ml. of these cells were washed by re-suspending them twice in fresh saline solutions. All the saline washings were pooled. In a third tube, the same volume of saline-washed cells was lysed with distilled water. The ghosts were washed with distilled water until no further radioactivity could be removed from them. The lysing solution containing the cell contents was added to the distilled water wash for measurement of the radioactivity of the cells exclusive of that bound to the stroma.

Separated organs and tissues were ashed and prepared for counting as previously described (Boroughs, Townsley and Hiatt, 1956). Radioactivity was measured with a thin window G-M tube using a commercial scaler. Counts were corrected for coincidence whenever necessary.

In order to get an approximation of mixing time, Sr^{85} was injected in the heart. Ten, 20, and 30 minutes later, blood was removed from the ventral aorta and from the kidney sinus, and 0.1-ml. samples were counted in a well scintillation counter with the aid of a single channel pulse height analyzer.

Results and Discussion

Preliminary experiments

Since very little is known about fish blood, we were at the outset faced with problems which were not pertinent to the main idea of this research. The first problem to be overcome was the bleeding, because apparently very few biologists have successfully removed blood directly from teleost fish (Prosser, personal communication). In general, fish have been bled by cutting the tail and allowing the blood to drip. Even more refined methods have involved the use of heparin, citrate, or other anticoagulants. We have found it difficult to withdraw unclotted blood from *Tilapia* if the fish had been kept out of water for even a short time. There is probably a dehydration of the blood in some species of fish as a result of asphyxiation (Hall, Gray and Lepkovsky, 1926). If *Tilapia* were stressed by prolonged chasing with a net, by rough handling or by repeated bleeding, removal of blood was difficult even though they were not taken from the water. The cell/plasma ratio increased as it did with asphyxiation.

We had previously observed red blood cell counts which varied between 1 and $4 \times 10^6/mm^3$ in this species of fish, and other workers (Young, 1949) have observed similar large variations with other teleost fishes. Table I is a summary of the rbc counts of the fish used in this experiment and shows that these variations are not intrinsic and that it is possible to remove fish blood that has a reasonably small fluctuation in the rbc count. This blood does not clot even on prolonged standing at room temperature.

The tremendous shift in the number of red blood cells observed in fish blood

TABLE I

Red blood cell count in Tilapia mossambica

Time interval between injection and killing	RBC/mm.³ of blood	
	Counted before dose injected	Counted before blood withdrawn
5 min.	1.444×10^6	1.627×10^6
5 min.	1.150×10^6	1.423×10^6
5 min.	1.375×10^6	1.400×10^6
15 min.	1.350×10^6	2.050×10^6
15 min.	1.209×10^6	1.374×10^6
30 min.	1.548×10^6	1.525×10^6
30 min.	1.175×10^6	1.400×10^6
45 min.	1.125×10^6	1.460×10^6
1 hr.	1.200×10^6	1.600×10^6
1 hr.	1.162×10^6	1.384×10^6
1 hr.	1.050×10^6	1.025×10^6
2 hr.	1.223×10^6	1.347×10^6
2 hr.	1.148×10^6	1.326×10^6
2 hr.	1.150×10^6	1.220×10^6
4 hr.	1.151×10^6	1.169×10^6
4 hr.	1.209×10^6	1.137×10^6
8 hr.	1.199×10^6	—
8 hr.	1.011×10^6	1.102×10^6
1 day	1.312×10^6	1.396×10^6
1 day	1.649×10^6	1.598×10^6
2 days	1.100×10^6	1.199×10^6
2 days	1.298×10^6	1.298×10^6
4 days	1.103×10^6	—
4 days	1.271×10^6	1.362×10^6
8 days	1.150×10^6	1.273×10^6
8 days	1.018×10^6	1.175×10^6

could mean that the plasma, or some portion of it, either leaves the circulatory system or is in effect removed by some pocketing device. The increase in red blood cells may also result from the introduction into the blood stream of cells previously sequestered in an organ or tissue. Studies on fish blood volume and mixing time using either classical techniques or radioisotopes would be of little value if the fish were stressed.

The circulation of fish blood is distinguished from that of higher animals in that oxygenated blood does not necessarily return to the heart. All the blood from the heart goes to the gills, but from the gills the blood may go to the head,

TABLE II

The mixing time of Tilapia blood

Blood source	Minutes elapsed	Counts/min.
Ventral aorta	10	249
Ventral aorta	20	79
Ventral aorta	30	51
Kidney	10	40
Kidney	20	45
Kidney	30	50

Dose: 8477 cpm in 0.2 ml. injected into ventricle of heart.

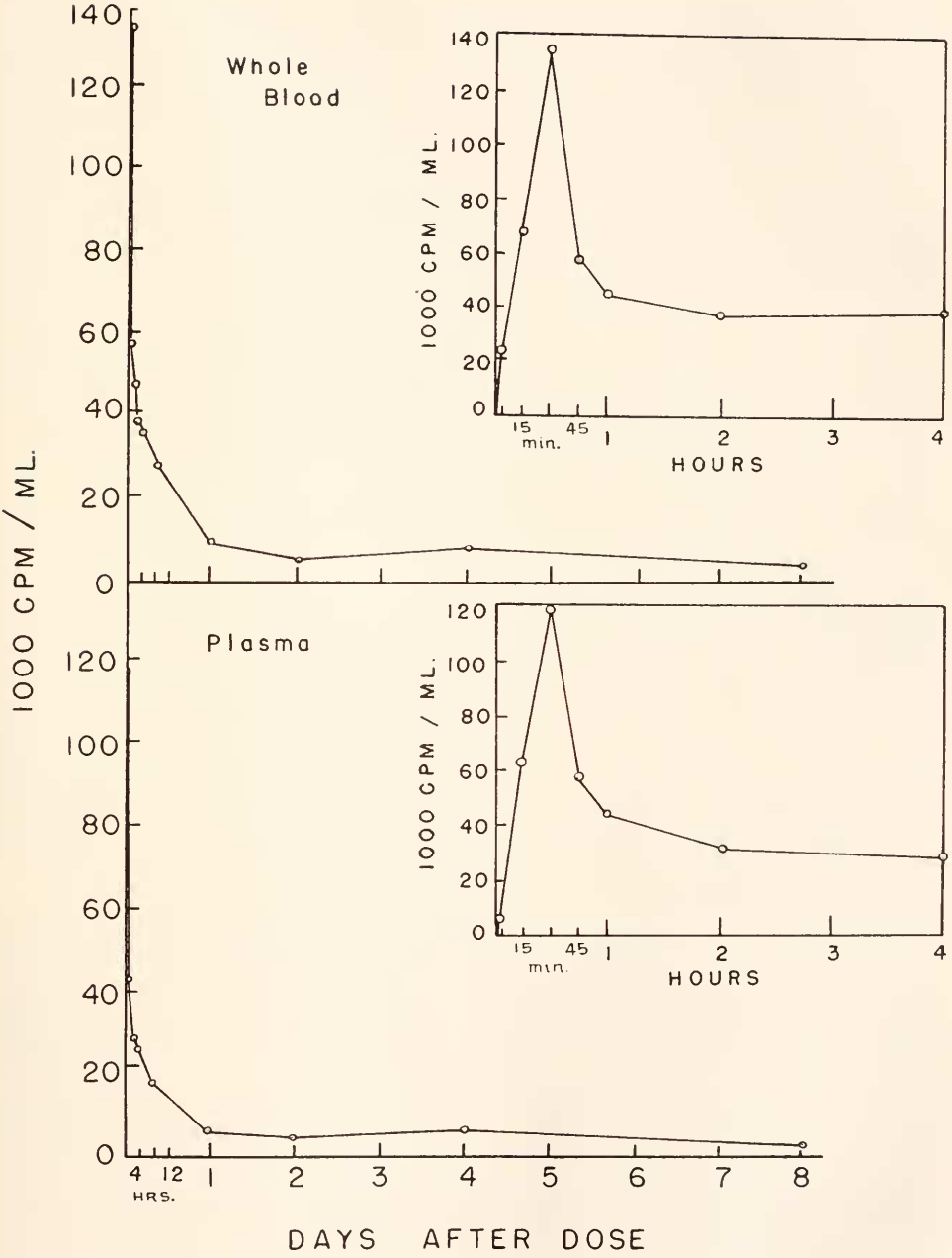

FIGURE 1. The disappearance of Sr^{90}–Y^{90} from the whole blood and plasma of *Tilapia mossambica*.

back to the heart, or to the remainder of the body. This means that mixing is a more complicated process in fish than it is in the higher animals.

The results of studying mixing time in a single fish are shown in Table II. It can be seen that the bulk of the Sr^{85} injected into the heart remained in the anterior portion of the fish, and that it required about 30 minutes for the blood from the ventral aorta and that from the kidney to reach the same level.

Since we lack precise information about blood volume, we have assumed that it is roughly proportional to body weight. We have done this not only on the basis of our own work, but because Martin (1950) has suggested a similar relationship for other teleost fishes.

Rate of disappearance of Sr^{90}-Y^{90} from the blood

Figure 1 shows the rate of disappearance of Sr^{90}-Y^{90} from whole blood and plasma. The numbers have been corrected for body weight. The activity is given in counts/min./ml. whole blood and cpm in the plasma present in 1 ml. of whole blood. Each point on the curve represents the average activity from at least two fish. It can be seen that practically all the radioactivity in the whole blood is carried in the plasma, and that the formed elements can be responsible for only a very small amount. The two curves are practically superimposable. The small inserts on this graph show the appearance of radioactivity during the first few hours, and the larger graph extends the curves to 8 days. Since all the radioactivity was injected into the heart at zero time, at first glance it may seem odd that the amount of radioactivity recoverable from the blood *increases* up to 30 minutes. However, Table II indicates that this apparent increase is a reflection of the mixing time. At least two processes are occurring during this time which make it extremely difficult to find out exactly how much radioactivity is in the blood system. First, the isotopes are being excreted as soon as they appear in the blood, at first principally by way of the gills. Second, radioactivity is rapidly accreted by the various organs and tissues, and thus the concentration is decreasing continuously. We would like to emphasize that it is the resultant of these processes that is being measured.

The radioactivity was very rapidly lost from the blood during the next 30 minutes, and after 24 hours, only between 0.8 and 1.6 per cent of the injected dose remained in the blood, assuming a blood volume of 2–4 per cent of the body weight. The shape of the curves shows that more than one rate process is involved in the disappearance of the radioactivity from the blood. It must be emphasized at this point that the above samples were counted at least three weeks after the fish was killed, so that we were observing the radioactivity in an equilibrium mixture of Sr^{90}-Y^{90}. Strontium^{90} has a half-life of about 28 years and a maximum beta energy of 0.61 Mev. It decays to form radioactive Y^{90} which has a half-life of 2.54 days and a maximum beta energy of 2.18 Mev. Secular equilibrium exists when the Y^{90} decays as fast as it is formed, and the radioactivity of such a mixture is the sum of the radioactivity of the separate isotopes.

In an equilibrium mixture, therefore, no decay of radioactivity would be observable during this experiment unless the two isotopes were separated by either biological or physico-chemical processes. Such a fractionation can be detected by following the counting rate of a sample daily. No changes in this rate will be

observed if no fractionation has occurred. If the rate increases, Y^{90} has been removed and is building up to its equilibrium value at which point it will level off. If the rate decreases, the bulk of the radioactivity must be due to the Y^{90} which is decaying, and the counts will decrease until a level is reached which is a function of the amount of Sr^{90} present.

The role of the blood fractions in the transport of Sr^{90}-Y^{90}

The increase in the counts/minute of the whole blood and plasma in Figure 2 is due to the build up of Y^{90}. There are two simple explanations for the loss of yttrium from the blood. One is that the yttrium was lost prior to its appearance in the blood initially, that is, adsorbed to the glassware used in making the dilutions

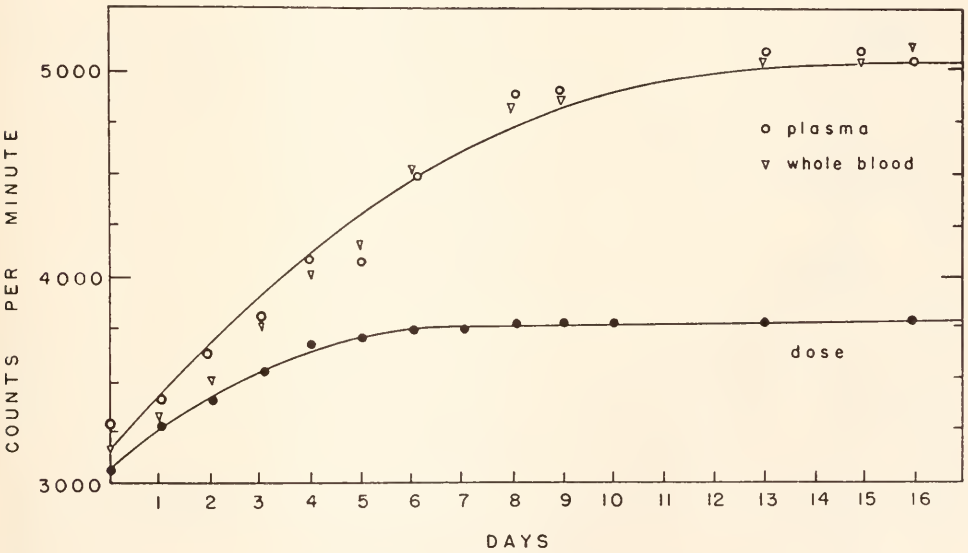

FIGURE 2. The increase with time of radioactivity in samples of whole blood, plasma, and the dose, indicating the build-up of Y^{90}.

and injections. The second explanation is that the yttrium was lost to various organs and tissues through which the blood passed. These explanations are not mutually exclusive and we believe that both processes occur.

In Figure 3, the curve labelled "dose" was obtained by counting planchettes prepared from the Sr^{90}-Y^{90} present in the syringe used for injections. It can be seen that over a period of time, the cpm increased, indicating that some Y^{90} was lost from the equilibrium mixture. This Y^{90} was lost to the glassware. The curve for whole blood and plasma, however, increased to a much higher value, indicating that additional Y^{90} had been removed after the dose was injected.

Figure 3 shows the rate of radioactive decay of the washed and unwashed cells, the saline washings, the washed ghosts, and the distilled water washings which include the cell contents. The decay of the unwashed cells suggests that both Sr^{90} and Y^{90} were associated with the cells. The decay of the washed cells, saline

wash, and ghosts, however, suggests that the Sr^{90} is readily removable either from or through the cell wall. The activity remaining in the washed cells and ghosts indicates it to be Y^{90}, because the decay rates are very similar to the rate for pure Y^{90}. All these conclusions are in harmony with the findings of Thomas *et al.* (Thomas, Litovitz, Rubin and Geschickter, 1950), who showed that radiocalcium, metabolically similar to strontium, was carried in the plasma of rabbit blood.

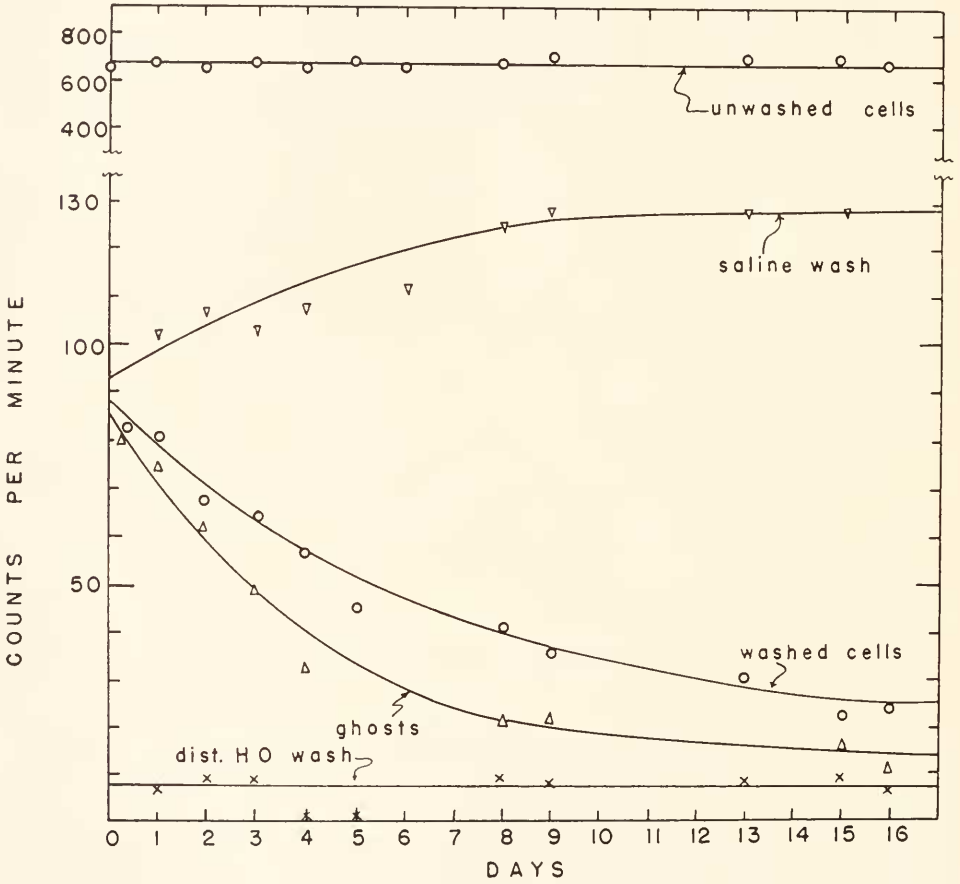

FIGURE 3. The radioactive decay of washed and unwashed cells, the saline and distilled water wash, and the cell ghosts. The decay of washed cells and ghosts indicates that they pick up Y^{90} rather than Sr^{90}.

Retention and distribution of Sr^{90}-Y^{90}

Figure 4 shows the retention by the fish of the injected Sr^{90}-Y^{90} as a function of time. The upper curve represents the entire fish, and the other curves represent, respectively, the bone, integument, gills, muscle, and visceral organs. Each point is the average of at least two fish, and the samples were counted at secular equilibrium. These results may be compared with those obtained previously by

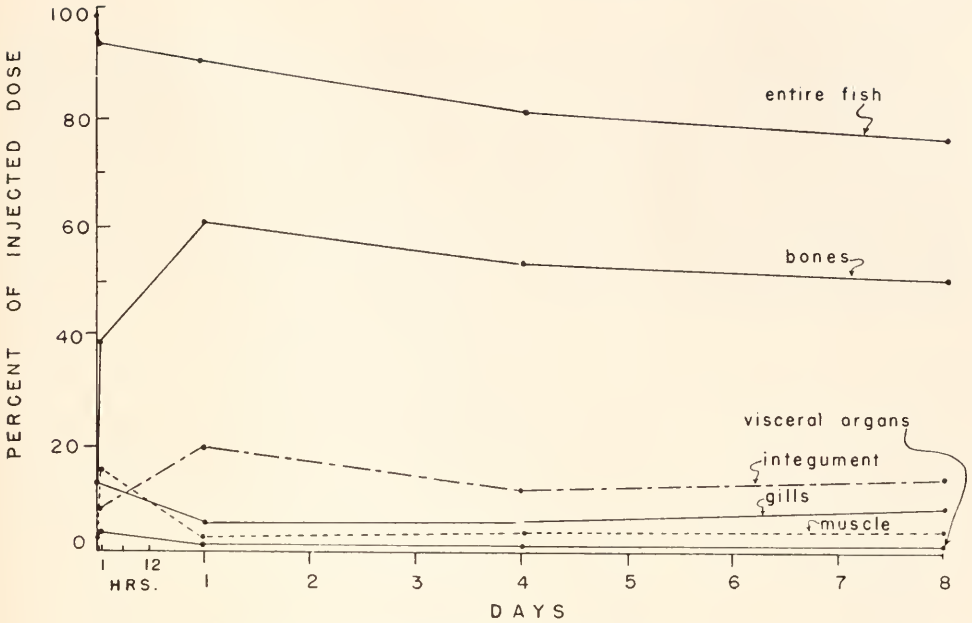

FIGURE 4. The internal distribution of Sr^{90}–Y^{90} injected in the heart of *Tilapia mossambica*.

Boroughs *et al.* on the retention of Sr^{89} by *Tilapia* following ingestion or intramuscular injection. In all instances, the rank order of the percentage of dose retained is the same as that in Figure 4, indicating that once the strontium is absorbed by the blood system, its internal distribution is the same.

Table III emphasizes the rapidity with which the radioactivity appears in the various tissues, including the bones. A small amount of vascular tissue and blood is dissected with the bones, but this additional radioactivity is obviously a very small percentage of the total in the bones. This appearance and retention in the bones cannot be due to accretion by growth, but must represent simply an exchange reaction.

TABLE III

Retention and internal distribution of Sr^{90}–Y^{90} after intracardial injection

Time interval	% injected dose remaining (samples at secular equilibrium)					
	Total of fish*	Bones	Integument	Gills	Muscle	Visceral organs
5 min.	98.2	13.2	13.4	13.4	3.1	3.4
15 min.	95.9					
30 min.	93.2	39.3	8.2	17.3	11.9	4.0
1 day	92.1	61.7	19.2	4.9	4.5	1.4
4 days	81.5	53.7	17.1	4.7	4.2	1.4
8 days	76.6	50.2	16.2	7.4	3.3	1.4

* Including blood.

Biological fractionation of Sr^{90}-Y^{90}

Three fish were injected with Sr^{90}-Y^{90} and killed five minutes, 30 minutes, and one day later. Since the amount of separation of the two isotopes by the glassware was unknown, it is not possible to draw a curve showing the rate of decay of the radioactivity in the various organs that would be a true measure of the decay due to the fractionation by the organs themselves. The planchettes were counted one day after the fish were killed, and this value was taken as a base line. They were then counted until secular equilibrium had been re-established. Table IV shows the percentage increase or decrease in radioactivity in the various organs with respect to the radioactivity present at one day.

TABLE IV

Fractionation of intracardially injected Sr^{90}–Y^{90} by organs and tissues of Tilapia mossambica

Organ or tissue sample	% Decrease of activity 1 day to secular equilibrium	Organ or tissue sample	% Increase of activity 1 day to secular equilibrium
Liver	53.1	Gills	73.8
Gall bladder	42.5	Stomach	22.3
Heart	38.6	Brain	18.3
Kidney	18.8	Muscle	16.3
Spleen	10.6	Intestine	10.4
Gonads	8.5	Eyes	6.9
		Urinary Bladder	5.6
Urine	28.5	Skin	5.0
Blood clots	26.8		
Scales	14.1		
Fat	11.4	Feces	2.1

It can be seen that the first two columns represent the organs which concentrated Y^{90} more than they did Sr^{90}, while the last two columns represent organs that favored the Sr. In general, the more vascular organs and tissues preferred yttrium.

SUMMARY

1. Blood can be easily removed without clotting from the heart or kidney sinus of fishes if the fish are handled gently and their opercula are kept immersed.

2. Blood so removed has a uniform number of red blood cells/mm^3.

3. The mixing time of Sr^{90}-Y^{90} injected in the ventricle of *Tilapia mossambica*, a teleost fish, is approximately 30 minutes.

4. Sr^{90}-Y^{90} rapidly disappears from the blood. At 24 hours, only between 0.8 and 1.6 per cent of the injected dose remains in the blood.

5. The disappearance of radioactivity from the blood depends on more than a single process.

6. Almost all of the Sr^{90} in whole blood is carried by the plasma.

7. Very little Sr^{90} is found either in the cells or on the cell walls.

8. $Yttrium^{90}$, on the other hand, is present in the stroma.

9. The pattern of internal distribution of intravascularly injected Sr^{90}-Y^{90} is the same as that which was found for either intramuscular or oral administration in the same species.

10. Vascularized tissues have a greater avidity for Y^{90} than they have for Sr^{90}.

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